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Renal Pharmacokinetic Changes of Gentamicin during Enterococcal Pyelonephritis

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In the present study, the renal pharmacokinetics of gentamicin was investigated in pyelonephritic rats infected with *Streptococcus* (*Enterococcus*) *faecalis*. Four days after the induction of infection, animals were given either a single dose of gentamicin or two daily injections for 7 days. The treated animals were evaluated from 1 h to 6 months posttreatment. After a single injection, limited pharmacokinetic variations were observed, whereas after 14 injections infected kidneys demonstrated significantly higher concentrations and a more extended renal elimination phase of the antibiotic. Analysis of the area under the curve of the concentration in kidney versus time showed more marked renal accumulation by the infected and long-term-treated animals than by normal animals or those receiving only one injection of aminoglycoside. Renal function remained normal in both the infected and normal animals treated with this aminoglycoside. These results demonstrate that *S. faecalis* pyelonephritis disturbs the renal handling of gentamicin and may increase the susceptibility of the kidney to aminoglycosides.

There are limited data on the intrarenal pharmacology of antibiotics in infected individuals, most studies having been done in healthy humans or animals (6, 14). We have observed in the past that renal infection did modify the pharmacokinetics of antibiotics. While infection reduced the amount of ampicillin in *Escherichia coli*-infected kidneys (25), aminoglycosides accumulated to a much greater extent within the infected cortex and medulla than in normal rat kidneys (7). Further investigations in this laboratory revealed that gentamicin was more nephrotoxic in pyelonephritic rats infected with *E. coli* than in noninfected animals (1). The mechanisms by which pyelonephritis modifies the intrarenal distribution of antibiotics and raises the nephrotoxic potential of aminoglycosides are not known. However, certain evidence led us to believe that bacterial endotoxins may largely contribute to the pharmacodynamic changes observed in gram-negative infections. Indeed, endotoxins are known to stimulate the mechanisms involved in the inflammatory process which have potent effects on vascular tone and permeability (20). Recently, we have shown that animals receiving low doses of lipopolysaccharide accumulated more aminoglycosides within the renal parenchyma than normal rats (5). Furthermore, some similarities between the nephrotoxic potential of gentamicin and that of bacterial lipopolysaccharide at subcellular and molecular levels suggest to us that there could be a synergistic toxic interaction between these molecules. These include their affinity for phospholipid membranes (21, 23), their direct action on lysosomes (15, 24) and on mitochondrial membranes, and the transport mechanisms (17, 22). In this study, renal pharmacokinetics of gentamicin was investigated in pyelonephritic rats infected with *Streptococcus* (*Enterococcus*) *faecalis*, a gram-positive coccus lacking endotoxin.

MATERIALS AND METHODS

Experimental animals. In this study, 350 female Sprague-Dawley rats weighing 175 to 225 g were used. Changes in animal weight and the amount of food and water absorbed were monitored during all the experiments. They were fed a standard rat diet free of antibiotic.

Test organism. A strain of *S. faecalis* ATCC 23241 (Lancefield group D) was used. The bacterium was isolated from the urine of a pyelonephritic patient (10). The gentamicin MIC and MBC for the strain were 32 and 128 μg/ml, respectively. Inoculum was prepared by subculturing the organism on blood agar, resuspending it in brain-heart infusion, and allowing growth until 10^7 to 10^8 CFU/ml was reached.

Experimental pyelonephritis. Shortly before each experimental period, the control and treated animals were weighed and anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Pyelonephritis was induced by two medullary inoculations of 0.05 ml of the inoculum.

Bacterial enumeration. The numbers of bacteria per milliliter of urine and per gram of kidney were determined from 24 h to 6 months postinfection. Groups consisted of 9 to 13 rats. After appropriate dilution of urine samples and homogenized kidney tissue (3), samples were placed in each of three pour plates containing blood agar and incubated for 18 to 24 h at 37°C. The evaluation of CFU of *S. faecalis* in urine and kidney samples by this technique allowed us to detect a minimum of 10 CFU/ml of urine and 30 CFU/g of kidney tissue. Kidneys and urine were considered sterile if no organisms were detected by this method. Results are expressed as mean log number of CFU per gram or milliliter ± standard error of the mean (SEM).

Aminoglycoside therapy. Treatment was started 4 days after inoculation of bacteria. Gentamicin (Schering Corp., Kenilworth, N.J.), 10 mg/kg, was administered intraperitoneally. Each rat received either a single dose or two daily injections administered every 12 h for 7 days. Groups of up to 12 rats were sacrificed from 1 h to 6 months after the end of therapy.

Aminoglycoside assay. Concentrations of gentamicin were evaluated at all timed intervals in the serum, cortex, medulla, papilla, and urine by a standard microbiological assay, with *Bacillus subtilis* as the test organism (4). Standard curves were prepared with either serum, cortex, medulla,

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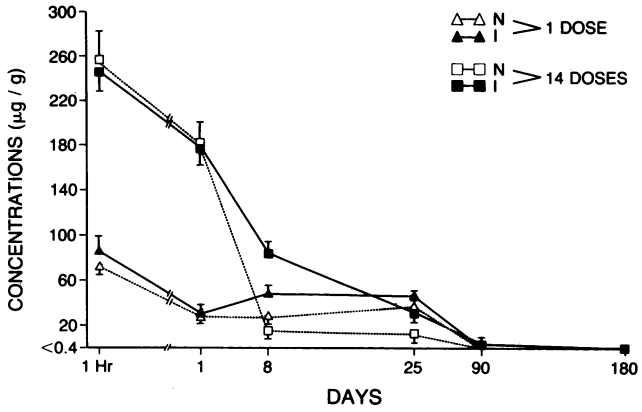

FIG. 1. Concentrations of gentamicin in normal (N) and right infected (I) cortexes after 1 or 14 injections of gentamicin (10 mg/kg of body weight).

papilla, or phosphate buffer for urine. The area under the curve of drug concentration versus time (AUC) was calculated by a standard method (18). The level of recovery of gentamicin after a known amount of this drug was added to antibiotic-free homogenates was 98 ± 1.6%.

Renal function. Serum creatinine and blood urea nitrogen levels were determined with an analyzer (ABA-100; Abbott Laboratories).

Statistical analysis. Data are presented as the mean ± SEM. The statistical differences were established by orthogonal comparison at 1 degree of freedom and by a Duncan multiple-range test.

RESULTS

Pathological effects. At 24 h after surgery, only 5 to 10% of animals were visibly ill; these exceptions demonstrated an asthenic and febrile state, but all animals recovered within 7 days following infection. Although the animals were apparently healthy, observation of the kidneys at sacrifice demonstrated a different situation. On day 4 after infection, the left kidney exhibited signs of pyelonephritis with inflammation and cortical abscess formation. Less severe ascending pyelonephritis with slight swelling and few inflammatory cells was observed in the right kidney. Six months later, the left kidney demonstrated a fibrous appearance and was slightly atrophied. However, inflammation signs were noted to a limited extent.

Weight and food and water intake. Compared with normal animals, pyelonephritic rats did not show significant differences in either body weight or volume of food and water consumed.

Bacterial count. At 24 h after *S. faecalis* injection, the mean number of CFU in the kidney tissue ± SEM was 3.9 ± 0.2 and 7.4 ± 0.1 log CFU/g in the right and left kidneys, respectively, and 5.9 ± 0.3 log CFU/g in the urine. In the right kidney the number of bacteria was 3.6 ± 0.6 log CFU/g 1 week after initiation of infection; 1, 3, and 6 months later, these values were 2.9 ± 0.6, 2.9 ± 0.7, and 3.7 ± 0.7 log CFU/g. The left kidney directly inoculated with bacteria showed a mean value of 5.9 ± 0.3 log CFU/g 7 days after the injection; 1, 3, and 6 months later, the bacterial counts were 5.4 ± 0.2, 3.8 ± 0.9, and 5.0 ± 0.3 log CFU/g. At 6 months, none of the left kidneys were sterile but 20% of the right kidneys had less than 30 CFU/g.

In the urine, the count was 4.7 ± 0.2 log CFU/ml 1 week after infection. During the following 1, 3, and 6 months, the mean values were 4.5 ± 0.3, 3.1 ± 0.6, and 4.7 ± 0.7 log CFU/ml. Twenty percent of specimens were sterile at 3 and 6 months, even though 100% of the left kidneys were infected.

Gentamicin treatment had no effect on bacterial counts, which were identical whether or not the animals were treated.

Gentamicin concentrations. Animals who received one dose showed similar peak levels in serum (normal, 9.7 ± 0.7 μg/ml, and infected, 11.6 ± 1.6 μg/ml) at 1 h. This was not the case for the long-term-treated rats, of which infected groups showed higher ($P < 0.01$) peak levels in serum (20.6 ± 2.9 μg/ml) compared with the normal group (13.0 ± 0.5 μg/ml). At 4 h, aminoglycoside concentrations were under the detection limit in all groups (<0.4 μg/ml). At 1 h and on days 8 and 25 after a single dose of gentamicin, concentrations of the antibiotic in the cortex were higher in the infected than in the control group ($P < 0.05$) (Fig. 1). The same phenomenon was observed in the papilla but at 1 h only, while in the medulla (Fig. 2) no significant change was observed, and at 25 days gentamicin was undetectable in this tissue. No significant differences in drug concentration were observed between the left and right kidneys.

Pharmacokinetic changes became more apparent after 7 days of treatment. Pyelonephritic rats showed higher renal drug concentrations than normal rats during the late elimination phase. These changes appeared on day 8 in the cortex ($P < 0.01$) (Fig. 1) and on day 25 in the medulla ($P < 0.01$) (Fig. 2). Infected kidneys showed the presence of gentamicin up to 3 months after cessation of therapy, while antibiotic was undetectable in normal animals. The AUC of gentamicin concentrations in the kidneys after a single injection was identical for both infected and normal animals. However, 14 doses led to a higher uptake of aminoglycoside by both left and right infected kidneys (Fig. 3).

Levels of drug in the urine are shown in Table 1. During the first 4 h after antibiotic administration, concentrations of gentamicin were lower in the urine of infected animals than

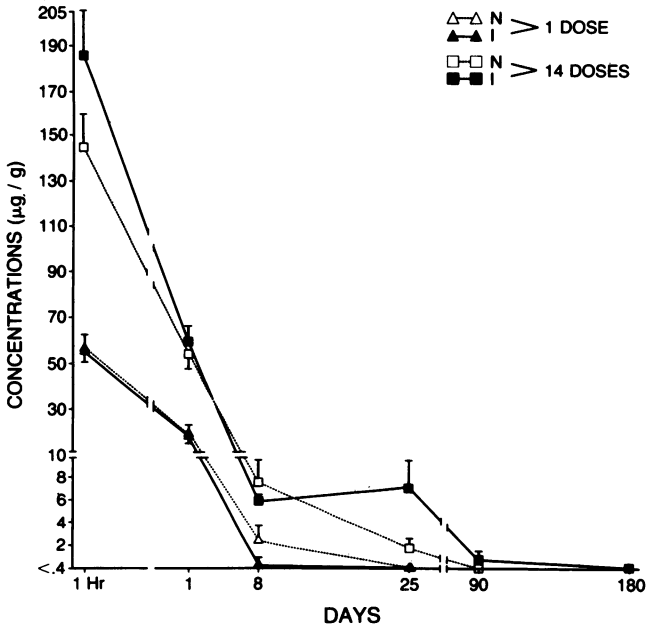

FIG. 2. Concentrations of gentamicin in normal (N) and right infected (I) medullas after 1 or 14 injections of drug (10 mg/kg of body weight).

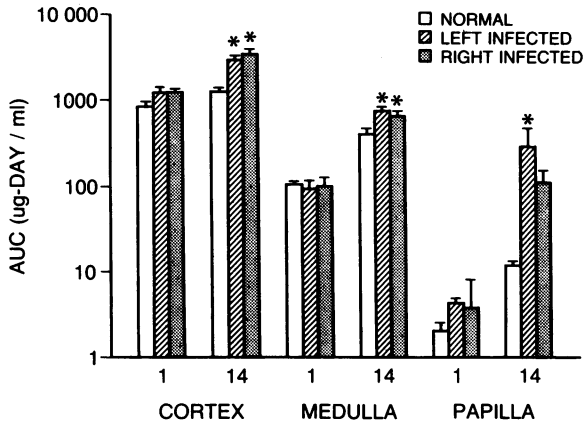

FIG. 3. AUC of gentamicin concentration versus time in normal, left infected, and right infected kidneys following either 1 or 14 injections of gentamicin (10 mg/kg). *, $P < 0.01$ versus normal animals.

in the normal group. Thereafter, in the animals treated for 7 days, in which we could still detect antibiotic, the concentrations were significantly higher in the infected animals than in the normal group (Table 1). After a single injection, the recovery rate from the urine was similar for both normal (91.1%) and infected (88.4%) animals. This was not the case for the 7-day treatment group, in which within 4 h of the last injection of drug, normal rats excreted 70.9% of the dose while infected rats excreted 35.2% ($P < 0.01$). Analysis of serum creatinine and blood urea nitrogen levels indicated that all groups had normal renal function at all times.

DISCUSSION

The results of the present study show that the pharmacokinetics of gentamicin is modified in animals suffering from *S. faecalis* pyelonephritis. Infected kidneys accumulated more gentamicin than normal kidneys. We have previously shown that kidneys infected with *E. coli* also had an increased uptake of aminoglycoside, but the changes observed in the presence of gram-negative infection were more severe. Furthermore, we observed modifications in the renal function of animals infected with *E. coli* (7), while in the present experiments, no modification in renal function was noted.

Gender is a factor that has to be considered in both human and animal experimentation. Data from Bennett et al. (2) show a significantly greater incidence of acute tubular necrosis in male than in female rats. On the other hand, Kourilsky et al. (12) observed a higher incidence of interstitial inflammation in female rats treated with gentamicin. Our study used female rats exclusively so that changes between groups are not due to sex differences. Moreover, we have never observed spontaneous pyelitis in untreated female rats. This study and previous ones (4, 7) used a female Sprague-Dawley rat pyelonephritis model. In fact, it was with this model that we first discovered evidence that infection might potentiate the nephrotoxicity of gentamicin (1). Moreover, recent reports by Kourilsky et al. (12) and Moore et al. (16) seem to suggest that human females have a higher incidence of aminoglycoside toxicity.

When compared with each other, antibiotics behave in a different manner in the presence of infection. In fact, tetracycline pharmacokinetics was not affected by *S. faecalis* pyelonephritis, but intrarenal levels were found to be diminished in animals suffering from gram-negative pyelonephritis (19). It was previously observed that the levels of ampicillin, cephalothin, and trimethoprim in the cortex and medulla were lower in *E. coli*-infected kidney parenchyma than in normal kidneys, while sulfamethoxazole levels were not affected by the presence of infection (3). While the previous observations lead us to believe that bacterial endotoxins largely contribute to the intrarenal pharmacokinetic changes observed in *E. coli*-pyelonephritic animals (5, 7), other mechanisms must be invoked to explain why *S. faecalis*, a bacterium without endotoxin, did influence the pharmacokinetics of gentamicin. Since gentamicin is reabsorbed in the proximal tubule, increased levels of the aminoglycoside in both serum and renal tissue were most likely the result of an underlying defect of excretion, which was most striking after a week of therapy with gentamicin. In fact, the infected animals excreted only 50% of the gentamicin recovered from the normal rats. One cannot exclude the possibility that the higher levels in serum observed on day 7 of treatment could be associated with greater tubular reabsorption and result in higher concentrations in the cortex. However, this does not explain why renal levels were increased after a single dose even if the serum levels were identical to those in the controls. Volume contraction in the animals which were infected for a long period (therapy with gentamicin being ineffective against *S. faecalis*) might explain the higher serum levels. Furthermore, we cannot eliminate the possibility that there might be an unapparent diminution of glomerular filtration rate that could contribute to elevated aminoglycoside levels in serum.

TABLE 1. Gentamicin concentrations in urine after 1 or 14 doses

Time after end of therapy	Mean gentamicin concn (μg/ml) ± SEM[a]			
	1 Dose		14 Doses	
	Normal rats	Infected rats	Normal rats	Infected rats
1 h	3,694 ± 201	2,641 ± 364	3,149 ± 352	2,140 ± 151*
2 h	2,791 ± 180	1,438 ± 289	1,250 ± 96	1,116 ± 170
4 h	424 ± 17	271 ± 54*	171 ± 43	97 ± 15
1 day	<0.4	<0.4	23.2 ± 6.3	42.1 ± 8.7
8 days	<0.4	<0.4	2.7 ± 0.5	17.1 ± 3.9**
25 days	<0.4	<0.4	0.6 ± 0.2	1.8 ± 0.1**
90 days	<0.4	<0.4	<0.4	<0.4
180 days	<0.4	<0.4	<0.4	<0.4

[a] Significance versus normal rats: *, $P < 0.05$; **, $P < 0.01$.

The influence of the pathological process of pyelonephritis on the dynamic interaction between aminoglycosides and kidney cells cannot be overlooked. In fact, the presence of active bacterial infection which results in renal inflammation (9, 11) might affect membrane permeability and transport of aminoglycoside in the kidney, resulting in more uptake.

However, in the right kidney, where the inflammatory process is less severe, the intrarenal pharmacokinetics is disturbed to the same degree. This suggests that the histopathological processes, such as cellular destruction and inflammation, associated with local infection are not solely responsible for the pharmacokinetic changes of gentamicin. In fact, stimulation of the arachidonic acid metabolism, like prostaglandin synthesis (M. E. Levison, P. G. Pitsakis, S. P. Levison, and B. Graves, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents. Chemother., abstr. no. 1164, 1986) and complement activation (13), may lead to hemodynamic and vascular permeability changes which could lead to disturbed distribution of aminoglycoside within the renal parenchyma. Pus, which is also known to bind aminoglycosides (8), could also partially contribute to the persistence of drug within the renal tissue. This factor surely did not play a determinant role for more than a few weeks after therapy, when the acute inflammatory process is slowly replaced by more chronic inflammatory cells and limited amounts of pus. Although the inflammatory response and cellular destruction are much less striking with gram-positive than with gram-negative bacteria (19), they did modify the dynamic interaction between kidney, bacteria, and aminoglycosides. These high levels of aminoglycoside may be beneficial if combined with an appropriate drug, like ampicillin, for the therapy of *S. faecalis* pyelonephritis. On the other hand, although we did not observe any modifications in renal function, the elevated intrarenal levels may increase the toxic potential of aminoglycosides.

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